Optimisation of the nutritional composition for the cultivation of *Eustoma grandiflorium* in hydroponics

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Subject category: Production of floricultural plants

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Index words: mixture theory, cation composition, anion composition, soilless cultures

ABSTRACT

The mineral composition of the nutrient solution was optimised for the cultivation of *Eustoma grandiflorum* on rockwool slabs. Both a cation and an anion experiment were carried out sequentially. In both experiments, 6 nutrient solutions were investigated with varying proportions of the macrocations (K^+, Ca^{2+} and Mg^{2+}) and the macroanions (NO_3^-, $H_2PO_4^-$ and SO_4^{2-}), respectively. All the nutrient solutions were formulated with the same microelement concentrations and a total salt concentration of 16 meq/litre. The effect of the mineral composition of the nutrient solution on the flower quality (stem length, stem weight, number of ramifications and number of flowering buds) of *Eustoma grandiflorum* was investigated. A high production of good quality flowers was obtained with the nutrient solution with the highest calcium proportion (3.52 mmol/l K^+, 5.28 mmol/l Ca^{2+} and 0.96 mmol/l Mg^{2+}). The highest stem weight per 100 cm and a high number of ramifications per flowering stem were obtained at a low dihydrogenphosphate and intermediate nitrate and sulphate proportions (11.04 mmol/l NO_3^-, 1.44 mmol/l $H_2PO_4^-$ and 1.76 mmol/l SO_4^{2-}). A second degree canonical polynomial was fitted to the response variables. The mathematical model obtained can be used to calculate the response for each cation or anion composition within the experimental region and to determine the direction with a maximum increase in response.

INTRODUCTION

Eustoma grandiflorum is a cut flower, for which an increasing interest in Belgian glasshouse floriculture exists. The crop cycle is relatively short (3 - 4 months). *Eustoma* is a facultative long day plant. Climatic conditions such as light intensity and temperature also influence the time of development of the flowering stem (Halevy, 1984). There is no information available about the nutritional requirements for the cultivation of *Eustoma grandiflorum* in hydroponics.

The ionic balance constraint imposed on nutrient solutions, postulates that the total amount of cation equivalents/litre equals the total amount of anion equivalents/litre. This is the major reason to define nutrient solutions as mixture systems (Schrevens, 1988; De Rijck, 1996). Experimenting with mixture systems in a classical orthogonal or in a unifactorial way, introduces confounding between the experimental factors (De Rijck and Schrevens, 1998). Due to this confounding, it is not possible to estimate the effect of the experimental factors.

Experimenting with mixture systems demands a specific experimental design and analysis of the experiments (Schrevens and Cornell, 1993). This mixture approach is used to optimise the cation and the anion composition of the nutrient solution for soilless culture to produce high quality flowers of *Eustoma grandiflorum*.

MATERIAL AND METHODS

Mixture systems

The properties of mixture systems are determined by the proportions of their components, rather than by their quantitative amounts. The proportion of each component must be nonnegative. If the proportions are expressed as fractions, they must sum to unity. A mixture system consisting of q components, with x_i the fraction of the ith component satisfies the following equations (Cornell, 1981,1990; Schrevens, 1988):

$$0 \leq x_i \leq 1 \qquad \text{for } i = 1, 2, 3, ..., q \tag{1}$$

$$\sum_{i=1}^{q} x_i = 1 \tag{2}$$

The q components of a mixture system are called “mixture variables”. The proportion of each mixture variable can vary from 0 (the component is not present) to 1, (a mixture with only one component). If in a q component mixture system the proportion of q-1 mixture variables is determined, then the proportion of the qth mixture variable is also determined:

$$x_q = 1 - \sum_{i=1}^{q-1} x_i \tag{3}$$

In this way equations (1) and (2) reduce the q dimensional factor space to a q-1 dimensional simplex (Claringbold, 1955). For a three component mixture system, the three dimensional factorspace is reduced to a two dimensional simplex (Figure 1). Each point in the simplex (equilateral triangle) represents a certain mixture, represented by its trilinear co-ordinates.

This reduction in dimensions has important consequences on the way to experiment with mixture systems. It is impossible to change the proportion of only one mixture component, without affecting the levels of the other components. This makes a unifactorial or an orthogonal way of experimenting with mixture systems impossible (De Rijck and Schrevens, 1994).

Nutrient solutions as mixture systems

Due to the ionic balance constraint (4), the amount of cations equals the amount of anions expressed in meq/litre. Simplifying a nutrient solution to the six essential macronutrients, this yields:

$$K^{+} + Ca^{2+} + Mg^{2+} = NO_3^{-} + H_2PO_4^{-} + SO_4^{2-} \qquad \text{(meq/litre)} \qquad (4)$$

Dividing the concentration of each cation (anion) by the total amount of cations (anions) expressed in meq/litre, yields the proportion of each cation (anion):

$$K^{+} + Ca^{2+} + Mg^{2+} = 1 \qquad \text{(proportions)} \qquad (5)$$

$$NO_3^{-} + H_2PO_4^{-} + SO_4^{2-} = 1 \qquad \text{(proportions)} \qquad (6)$$

In this way both the cation and the anion composition of the nutrient solution is determined as a 3 component mixture system. The mineral composition of a nutrient solution is determined by the cation proportions, the anion proportions and the total ionic concentration (De Rijck, 1996).

Experimental set-up

In both the cation and the anion factorspace, a {3,2}-simplex lattice design (Cornell, 1981,1990; Schrevens, 1988) was set-up, consisting of 6 experimental design points (Tables 1 and 2). The nutrient solutions were formulated at a total ionic concentration of 16 meq/l. Multiplying the proportion of each cation (anion) with the total ionic concentration expressed in meq/l, divided by its valence, yields its concentration in mmol/l.

Each nutrient solution contained also 30 µmol/l Fe, 20 µmol/l B, 28 µmol/l Mn, 0.4 µmol/l Cu, 0.5 µmol/l Mo and 1.2 µmol/l Zn.

Eustoma grandiflorum seedlings (pink Fujii selection) were grown in rockwool slabs in a computer controlled greenhouse. Planting density was 20 plants per meter gully length. The minimal air temperature was 16°C and ventilation temperature was 20 °C. Thermal screens (LS 15, Ludvig Svenson, Kinna, Sweden) were closed at irradiance exceeding 500 W/m².

Nutrient solutions were applied with drip irrigation, irrigation frequency was based on solar irradiation (threshold value 5 MJ/m²), and the average drainage percentage was 25-30%.

Cation experiment

Eustoma grandiflorum seedlings were planted on 30 March 1993. The crop cycle lasted 92 days. The average temperature was 19.9 °C, the irradiation sum for the crop cycle was 531 MJ/m^2. The 6 investigated cation compositions are represented in Table 1. The experimental region had a range of 7.04 meq/l in the K^+, Ca^{2+} and Mg^{2+} direction and was shifted towards a low magnesium proportion. The anionic composition was the same for all the nutrient solutions and was the same as solution 1 in the anion experiment (Table 2).

Anion experiment

The seedlings were planted on 21 February 1994. The crop cycle lasted 120 days. The average temperature was 19.4 °C, the irradiation sum for the crop cycle was 470 MJ/m^2.

The experimental region had a range of 4.16 meq/l in both the NO_3^-, $H_2PO_4^-$ and SO_4^{2-} direction and was shifted towards a high nitrate proportion. The cationic composition was the same for all the nutrient solutions and was the same as solution 1 in the cation experiment (Table 1).

Statistical analysis

In both the cation and the anion experiment, the 6 nutrient solutions were applied as a randomised complete block design with 3 replicates. Per replicate 20 plants were analysed.

Plant measurements were stem length, stem weight, ramification of the flowering stem and number of flower buds.

Plant data were examined by analysis of variance, mean separation was calculated by Duncan Multiple Range tests with 95 % confidence limits (SAS, 1991). If significant differences for a crop parameter between treatments were detected, the response was modelled by a second degree canonical polynomial consisting of 6 terms :

$$f(x) = \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1x_2 + \beta_{13} x_1x_3 + \beta_{23} x_2x_3 \quad (7)$$

with: x_1, x_2, x_3: proportion K^+, Ca^{2+} and Mg^{2+} or NO_3^-, $H_2PO_4^-$ and SO_4^{2-} respectively

x_1x_2, x_1x_3, x_2x_3: interactions between the respective ions

β_1, β_2, ...: parameter estimates

RESULTS AND DISCUSSION

Cation experiment

Analysis of variance

Flower stem quality was characterised by stem length, stem weight per 100 cm stem length, number of ramifications of the flowering stem and number of flower buds. The cation composition of the nutrient solution had no significant influence on the number of flowering buds (Table 3). Nutrient solutions 3 and 4 with a high Ca^{2+} and a high Mg^{2+} proportion respectively, yielded long flowering stems. The highest K^+ content in the nutrient solutions resulted in significantly the lowest weight per 100 cm stem. Nutrient solution 3 (high Ca^{2+}) resulted in the highest number of ramifications.

Regression

A second degree canonical polynomial was fitted to the stem length, the stem weight (g/100 cm) and the number of stem ramification (Table 4). Each of the models had a high adjusted R^2 and a relatively low coefficient of variation. The models were used to represent the response surface over the experimental region for the respective response variables.

The vertical axis in the figures represents the respective response variables. The triangular ground surface in the figures 2 – 6 represents the experimental region (Figure 1). At the right, the front and the back vertex of the simplex, the magnesium, potassium and calcium proportion are at their maximum (0.56, 0.66 and 0.66 respectively). Each point within the experimental region represents a certain cation composition as a function of its trilinear co-ordinates.

At a high calcium proportion (low potassium and low magnesium), the stem length was highest (Figure 2). Reducing the calcium proportion reduced the stem length in both the potassium and the magnesium direction. All the cations interacted antagonistic with each other for the stem length.

Both the calcium and magnesium proportion of the nutrient solution had a strong positive influence on the stem weight per 100 cm (Figure 3). Increasing the potassium proportion in the nutrient solution reduced the stem weight per 100 cm. While calcium interacted antagonistically with potassium and with magnesium, potassium interacted synergistically with magnesium for the stem weight per 100 cm.

A nutrient solution with a high calcium proportion yielded a high number of ramifications of the flowering stem (Figure 4). Reducing the calcium proportion decreased the number of ramifications and this mainly in the potassium direction. The cations interacted in the same way as for the stem weight per 100 cm.

Anion experiment

Analysis of variance

The anion composition of the nutrient solution had no significant effect on the stem length and the number of flowering buds (Table 5). The anion compositions 1, 6 and 2 (intermediate and high nitrate) yielded flowers with a high stem weight per 100 cm. Solution 5 (low nitrate and intermediate dihydrogenphosphate and sulphate) produced flowers with an inferior stem weight and significantly less ramification of the flowering stem.

Regression

For the stem weight (g/100 cm) and the number of ramifications, a second degree canonical polynomial was fitted to the data (Table 6). The obtained models were used to represent the response surface over the experimental region.

Nitrate, dihydrogenphosphate and sulphate had a similar effect on the stem weight per 100 cm (Figure 5). Due to the strong synergistic interaction between nitrate and sulphate, the stem weight per 100 cm was highest at a low dihydrogenphosphate proportion and intermediate nitrate and sulphate proportions. There existed a strong antagonistic interaction between dihydrogenphosphate and sulphate for the stem weight per 100 cm.

The number of ramifications per flowering stem was highest at a high nitrate proportion (Figure 6). Both a high dihydrogenphosphate and sulphate proportion resulted in a similar number of ramifications. Due to the strong antagonistic interaction between dihydrogenphosphate and sulphate, the number of ramifications was lowest at a low nitrate and intermediate dihydrogenphosphate and sulphate proportions.

CONCLUSION

The ionic balance constraint defines nutrient solutions as mixture systems. Experimenting with mixture systems demands a specific design and analysis of the experiments. To optimise the nutritional composition for the cultivation of *Eustoma grandiflorum* in soilless culture, a {3,2} simplex lattice design was set-up in both the cation (K^+, Ca^{2+} and Mg^{2+}) and the anion (NO_3^-, $H_2PO_4^-$ and SO_4^{2-}) factorspace.

Increasing the calcium proportion in the nutrient solution improved the quality of the flowers (stem length, stem weight, number of ramifications). Cation composition 3 (3.52 mmol/l K^+, 5.28 mmol/l Ca^{2+} and 0.96 mmol/l Mg^{2+}) yielded the best results.

The anion composition of the nutrient solution had no significant effect on the stem length and the number of flowering buds. A low dihydrogenphosphate and intermediate nitrate and sulphate proportions (11.04 mmol/l NO_3^-, 1.44 mmol/l $H_2PO_4^-$ and 1.76 mmol/l SO_4^{2-}), yielded the highest stem weight per 100 cm and a high number of ramifications per flowering stem.

If there exists no interactions between the cation and the anion composition of a nutrient solution, the combination of the best cation composition and the best anion composition forms the optimal nutritional composition. This optimal composition can shift as a function of other external variables (climate, irradiation, total ionic concentration, etc.).

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Table 1. Experimental design points of the {3,2} simplex lattice design in the cation factorspace

Design point	K^+		Ca^{2+}		Mg^{2+}	
	Meq/l	Proportion	Meq/l	Proportion	Meq/l	Proportion
1	7.04	0.44	7.04	0.44	1.92	0.12
2	10.56	0.66	3.52	0.22	1.92	0.12
3	3.52	0.22	10.56	0.66	1.92	0.12
4	3.52	0.22	3.52	0.22	8.96	0.56
5	3.52	0.22	7.04	0.44	5.44	0.34
6	7.04	0.44	3.52	0.22	5.44	0.34

Table 2. Experimental design points of the {3,2} simplex lattice design in the anion factorspace

Design point	NO_3^-		$H_2PO_4^-$		SO_4^{2-}	
	Meq/l	Proportion	Meq/l	Proportion	Meq/l	Proportion
1	11.04	0.69	1.44	0.09	3.52	0.22
2	13.12	0.82	1.44	0.09	1.44	0.09
3	8.96	0.56	1.44	0.09	5.60	0.35
4	8.96	0.56	5.60	0.35	1.44	0.09
5	8.96	0.56	3.52	0.22	3.52	0.22
6	11.04	0.69	3.52	0.22	1.44	0.09

Table 3. Effect of the cation composition of the nutrient solutions on the response variables

Design point	Stem length (cm)	Stem weight per 100 cm (g)	Ramifications flowering stem	Flowering buds
1	88.05 B*	80.57 C	2.33 AB	7.98
2	89.13 B	73.36 D	2.20 B	7.90
3	93.37 A	90.45 A	2.50 A	8.22
4	90.80 AB	87.05 ABC	2.33 AB	8.35
5	87.70 B	82.54 BC	2.20 B	8.10
6	88.63 B	89.22 AB	2.38 AB	7.78

*Mean separation within columns by Duncan multiple range test, $P<0.05$

Table 4. Second degree model to represent the response variables as a function of the cation composition of the nutrient solution

Variable	Stem length	Stem weight per 100 cm	Ramification flowering stem
K	98.09	45.00	1.73
Ca	115.28	121.59	3.24
Mg	110.02	79.62	2.70
KCa	-66.11	-27.65	-0.34
KMg	-27.55	186.29	2.41
CaMg	-90.55	-128.24	-4.48
Adj R^2	0.99	0.95	0.96
C.V.	8.99	22.33	21.08

Table 5. Effect of the anion composition of the nutrient solutions on the response variables

Design point	Stem length (cm)	Stem weight per 100 cm (g)	Ramifications flowering stem	Flowering buds
1	87.95	62.21 A*	3.25 A	12.95
2	88.52	57.47 AB	3.45 A	13.05
3	87.93	56.15 AB	3.23 A	11.87
4	87.0	55.50 AB	3.20 A	11.75
5	86.52	50.26 B	2.82 B	11.45
6	85.27	57.63 AB	3.23 A	12.25

*Mean separation within columns by Duncan multiple range test, $P<0.05$

Table 6. Second degree model to represent the response variables as a function of the anion composition of the nutrient solution

Variable	Stem weight per 100 cm	Ramifications flowering stem
NO_3	40.61	3.85
H_2PO_4	59.68	7.08
SO_4	-79.05	7.21
$NO_3H_2PO_4$	67.55	-5.42
NO_3SO_4	319.72	-5.42
$H_2PO_4SO_4$	-329.09	23.67
Adj R^2	0.90	0.92
C.V.	33.24	28.70

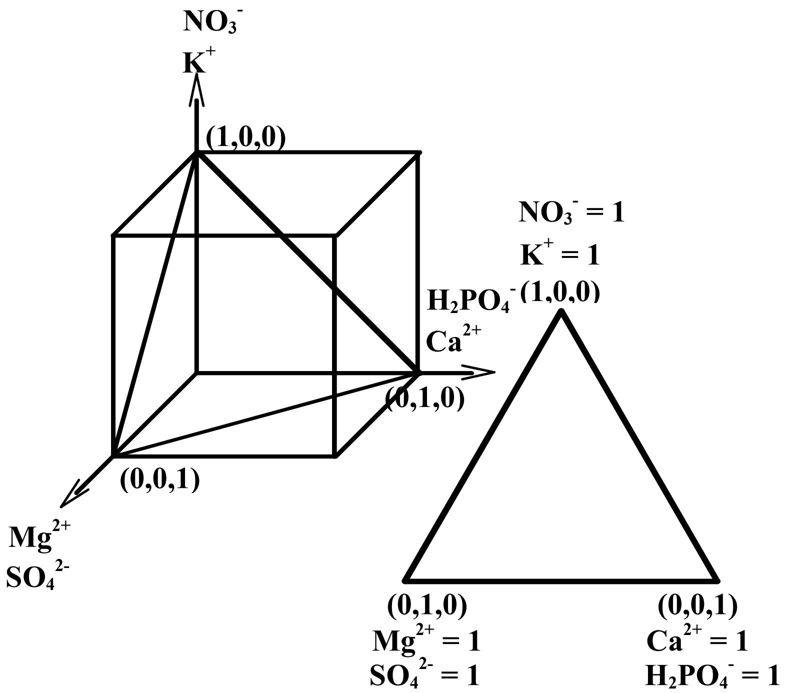

Fig. 1. Dimension reduction of the cation and the anion factorspace from a 3 dimensional cube to a 2 dimensional simplex

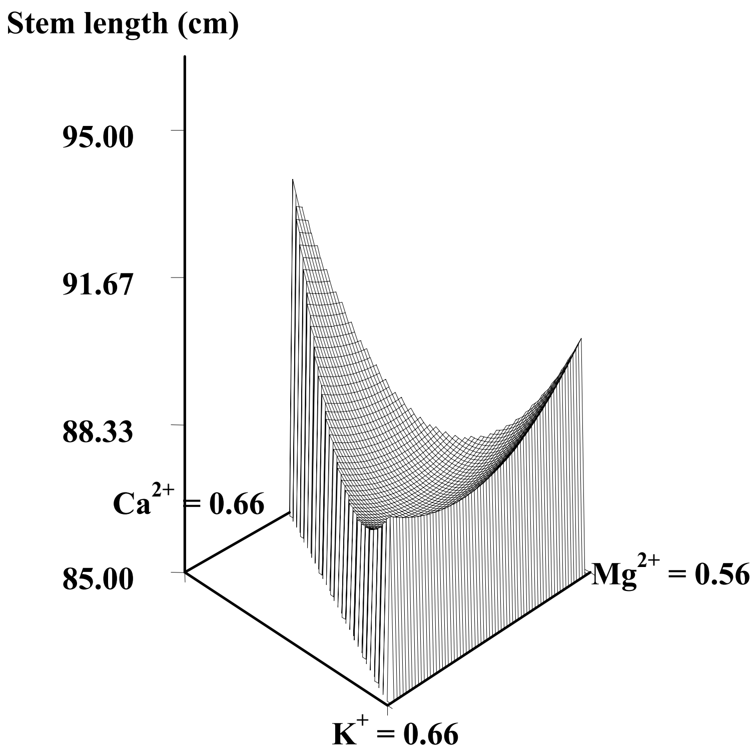

Fig. 2. Stem length (cm) as a function of the cation composition of the nutrient solution

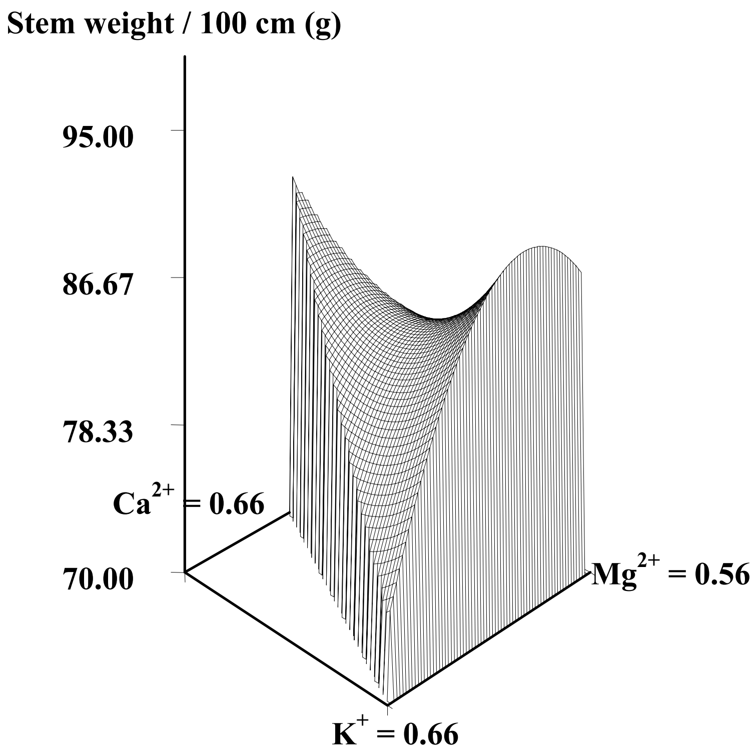

Fig. 3. Stem weight per 100 cm (g) as a function of the cation composition of the nutrient solution

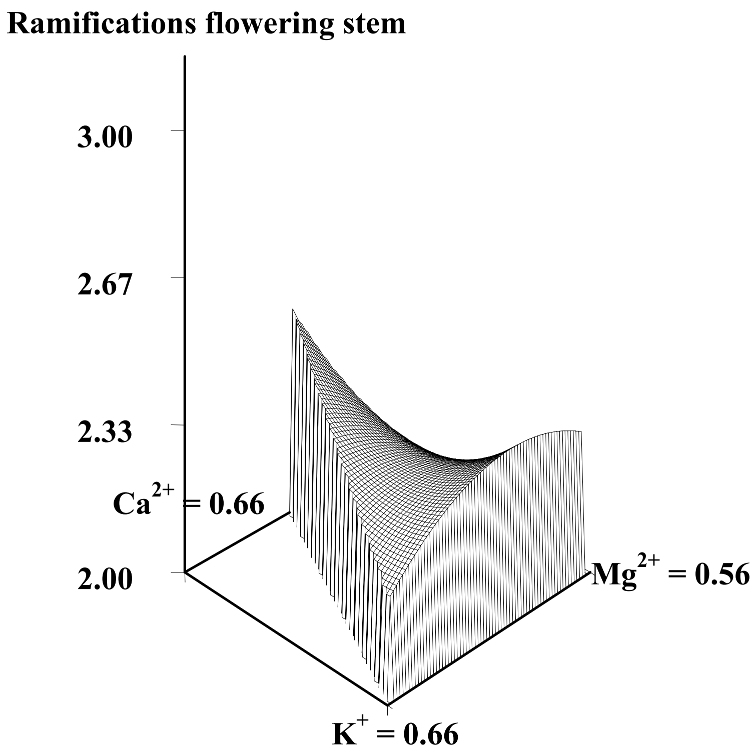

Fig. 4. Number of ramifications as a function of the cation composition of the nutrient solution

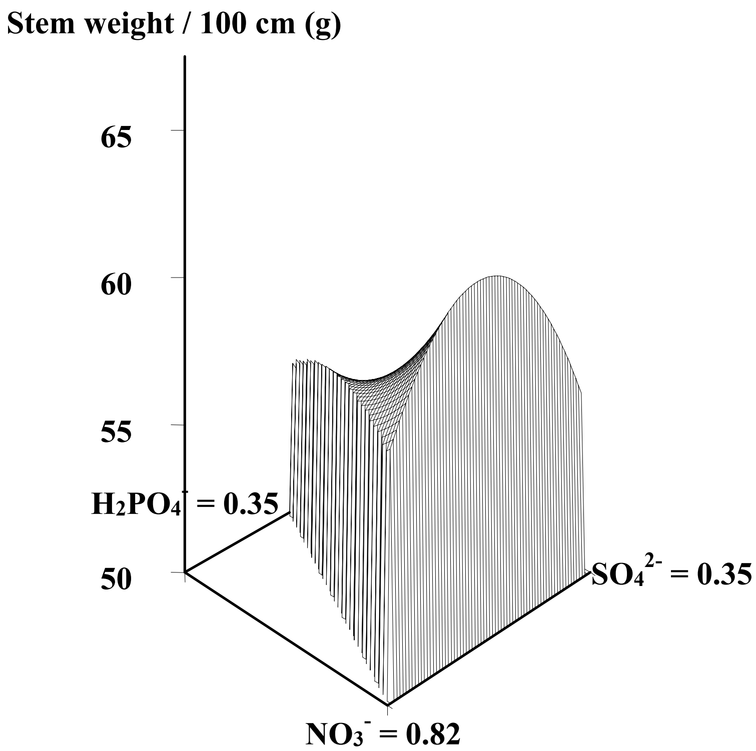

Fig. 5. Stem weight per 100 cm (g) as a function of the anion composition of the nutrient solution

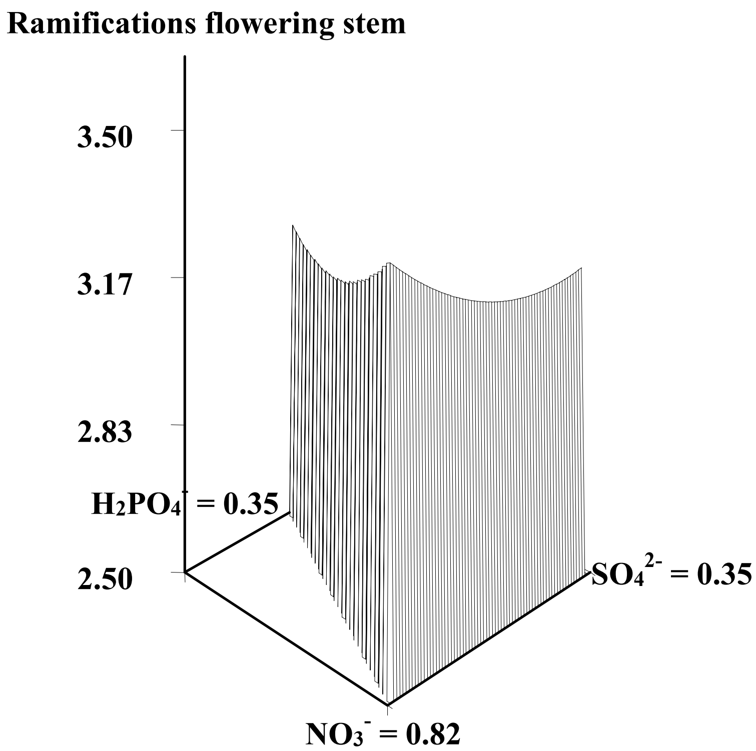

Fig. 6. Number of ramifications as a function of the anion composition of the nutrient solution